REMARKS

Applicants appreciate the notification that all of the previous rejections have been withdrawn.

Rejections Under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 10-16 as allegedly indefinite. The Examiner stated that both claim 10 and claim 16 have inconsistent limitations. According to the Examiner, recitation of primers "which flank the polymorphic site" is inconsistent with a primer having a "5' portion which, when incorporated into an amplification product, will upon further amplification yield products that form a stable stem-loop structure, the stem of which is perfectly matched and includes the polymorphic site".

The Examiner is mistaken. The primers flank the polymorphic site. Thus, the primers bind to either side of the polymorphic site, and neither primer extends over the polymorphic site. However, during amplification the primers are extended by the polymerase. Because of this the amplification products will, of course, include the polymorphic site. Accordingly, it is perfectly possible for a primer that flanks (does not include) the polymorphic site to be extended upon amplification to create an amplification product that does include the polymorphic site.

Contrary to the Examiner's assertion there is no contradiction between the claim requirement that the primers flank the polymorphic site and the claim requirement that "one of the two primers including a 5' portion which, when incorporated into an amplification product, will upon further amplification yield products that form a stable stem-loop structure, the stem of which is perfectly matched and includes the polymorphic site".

The Examiner is referred to the amendment filed November 15, 2001 which explains how the 5' portion of one of the primers becomes incorporated into an amplification product that upon further amplification yields products that form a stable stem loop structure. Applicants repeat this explanation below.

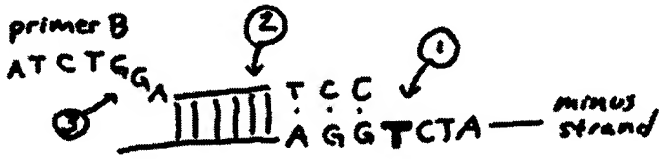
Consider a gene of interest that has a polymorphic site at a known position. The nucleotide at this position is "T" in the case of one allele ("the 'T' allele") and "C" in the case of

the other allele ("the 'C' allele"). A portion of the minus strand of the "T" allele is depicted below. The polymorphic "T" (1) is shown in **bold**. In the drawings only a few nucleotides in the area of the polymorphic site are explicitly depicted and some base-pairs are depicted by a vertical line.

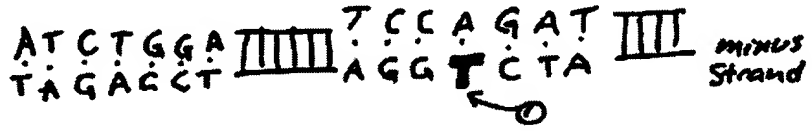
A subject is known or thought to be heterozygous at the this polymorphic site, and it is considered desirable to haplotype the patient by obtaining a relatively pure sample of the "C" allele. This can be achieved by using the presently claimed methods. In this instance specially designed primers that permit amplification of the "C" allele while inhibiting amplification of the "T" allele are used as follows.

A sample of DNA taken from the patient is subjected to PCR amplification using two primers. The first primer (Primer A) binds to the plus strand (not shown) and is a conventional primer. The second primer (Primer B) binds to the minus strand and is designed to incorporate sequences into the amplification product that cause the formation of a perfect (relatively stable) stem-loop structure upon amplification of one allele, in this case the "T" allele, and forms an imperfect (relatively unstable) stem-loop structure upon amplification of the other allele, in this case the "C" allele.

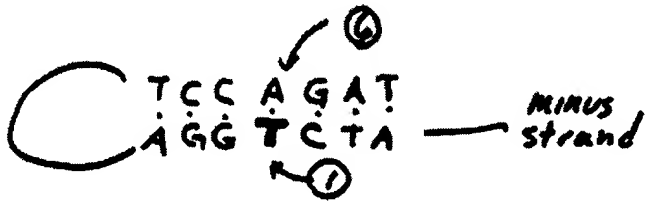
The sketch below depicts the initial binding of Primer B to the minus strand of the T allele. A 3' portion (2) of Primer B is complementary to the minus strand. A 5' portion (3) of this primer is <u>not</u> complementary to the minus strand, although after two rounds of amplification, this portion will be incorporated into the amplification product. The 5' portion that is not complementary to the minus strand (3) includes a sequence that will, during amplification, cause the incorporation into the minus strand of a sequence that can base-pair with the region surrounding the polymorphic site (1) thus forming the stem of a stem-loop.



After two rounds of amplification, the amplification product has the structure shown below. The 5' portion of the primer (3) has become incorporated into the amplification product, and it can be seen that the incorporation of the primer sequence has led to the incorporation into the minus strand a portion (4) that will form the stem of the stem-loop. This portion is perfectly complementary to the region surrounding the polymorphic site (1). Thus, it includes an "A" (5) that can base-pair to the "T" at the polymorphic site (1).

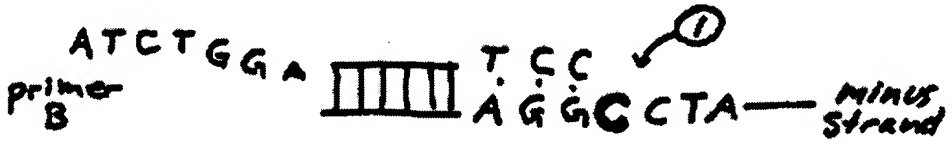


During the next round of denaturation and annealing, the minus strand of the amplification product forms a stem-loop structure, depicted below, having a perfectly matched stem (6). This stem includes the polymorphic site T (1). The stem-loop structure is stable enough to inhibit further amplification of the T allele relative to the C allele.



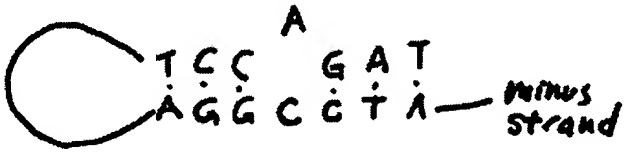
The situation is different for the C allele. This allele, depicted below, differs from the T allele in that a "C" is present at the polymorphic site (1).

As depicted below, Primer B binds to the "C" allele just as it binds to the "T" allele.



After two rounds of amplification, the 5' region of the primer is incorporated into the amplification product. Of course, in this case, the very 5' end of the minus strand is not perfectly complementary to the region surrounding the polymorphic site. This is because the primer was designed to be perfectly complementary to the region of the polymorphic site only when a "T" is present at the polymorphic site, not when a "C" is present.

Thus, upon the next round of denaturation and annealing, the stem of the stem-loop that forms is imperfect, as shown below. It contains a mis-match at the polymorphic site. This stem loop is not stable enough to significantly inhibit further amplification.



As a result, "C" allele nucleic acid molecules undergo additional amplification while amplification of "T" allele nucleic acid molecules is inhibited. As a result of the processes described above, amplification with Primer A and Primer B produces amplification product that is greatly enriched for copies of the "C" allele. This amplification product can be used to haplotype the "C" allele without excessive interference from the "T" allele.

With this understanding of the claimed methods it is clear that the claimed methods do not include steps that are inconsistent.

In view of the forgoing, Applicants respectfully request that the rejections under 35 U.S.C. §112, second paragraph be withdrawn.

Rejections Under 35 U.S.C. 102(e)

The Examiner rejected claims 10-16 as anticipated by U.S. Patent No. 6,361,940 to Van Ness et al. ("Van Ness"). Citing columns 59-60 of Van Ness, the Examiner argues that Van Ness discloses a method for biasing DNA amplification that entails the use of primers that "flank the polymorphic site such that both primers do not hybridize to the polymorphic site" and yield "extension products that incorporate perfectly matched nucleotides at the polymorphic site." Applicants disagree with both the Examiner's characterization of Van Ness and the Examiner's conclusion that claims 10-16 are anticipated.

First, Van Ness does not describe primers that flank the polymorphic site. At col. 59, line 19, Van Ness states that the "3' end of the primer is placed directly over the single nucleotide polymorphism." A primer having a 3' end that is directly placed over a polymorphism is clearly not a primer that flanks the polymorphism. Because Van Ness does not disclose primers that flank the polymorphic site, it cannot anticipate the present claims.

Second, Van Ness describes the use of two primers that have the identical sequence except for the fact that one primer matches the polymorphic site and the other does not (see col. 59, lines 29-31). Thus, it is not true, as the Examiner asserts, that neither primer described by Van Ness hybridizes to the polymorphic site. Moreover, a primer that extends over the polymorphic site does not flank the polymorphic site irrespective of whether the nucleotide extending over the polymorphic site.

Third, the Examiner has not cited any disclosure in Van Ness of a primer that upon amplification "yield products that form a stable stem-loop structure" as required by the claims. The Examiner certainly has not described such a primer that forms a stem loop, "the stem of which is perfectly matched and includes the polymorphic site only when the second nucleotide is present at the polymorphic site, but not when the first nucleotide is present at the polymorphic site." Because Van Ness does not disclose either of the these limitations, it cannot anticipate the present claims.

In view of the forgoing, Van Ness cannot anticipate the present claims, and Applicants respectfully request that the rejections under 35 U.S.C. 102(e) be withdrawn.

Enclosed is a Petition for Extension of Time with the appropriate fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

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